Performance evaluation of bulk freeze dried starter cultures of dahi and yoghurt along with probiotic strains in standardized milk of cow and buffalo

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Abstract Performance of bulk freeze dried (BFD) cultures of dahi (D) and yoghurt (Y) either with or without probiotic cultures (AB -*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) in standardized milk of cow and buffalo was evaluated. In buffalo milk, significantly ($p < 0.05$) low viable count of probiotic culture combination of dahi cultures (DAB) over non probiotic combination (D) was noticed; whereas, difference in counts of yoghurt culture combinations Y and YAB was not significant. The culture activity of D and DAB was similar in both types of milk, however, the volatile acidity (VA) produced by combination D was higher (32.5 ml/50 g sample) in buffalo milk than in cow milk (29.2 ml/50 g sample). Whereas, DAB produced very low amount of VA (16 ml/50 g sample) both in cow and buffalo milk. The diacetyl and tyrosine contents produced by either D or DAB in cow or buffalo milk were in the same order. Although Y and YAB produced slightly more VA in buffalo milk than in cow milk, significant change in the performance of yoghurt cultures (Y or YAB) both in cow and buffalo milk was not noticed. However, the VA and acetaldehyde produced by YAB either in cow and buffalo milk was higher than that by combination Y. Addition of probiotic cultures significantly enhanced the production of acetaldehyde content in both types of milk. Difference in tyrosine content in yoghurt made either with cow or buffalo milk was not significant. Overall, the present study indicated that the BFD cultures can be used to prepare dahi or yoghurt either from cow or buffalo milk, without affecting the biochemical profile of these products.

Keywords Acetaldehyde · Culture activity · Diacetyl · Proteolytic activity · Viable count · Volatile acidity

Introduction

Starter cultures are the key components in the preparation of fermented foods. The selection of starter cultures should be based on the consideration of their growth in association and other factors like competitive behaviour, inhibition of pathogens and spoilage microorganisms, their biochemical performance, probiotic properties, reduction of antinutritional factors etc. (Holzapfel 1997). Commercialization of traditional fermented foods leads to the production of fermented foods in large quantities. Initially dairy starter cultures are propagated on daily basis and later these are available as concentrates either in frozen or freeze dried form (Sandine 1996). Freeze drying is the commonly used method to prepare the dried starter cultures (Carvalho et al. 2003; Santivarangkna et al. 2007) and probiotic cultures (Santivarangkna et al. 2011a).

Maintaining the pure starter cultures and preparation of the cultures for large volume inoculations in the industry atmosphere, although may not be impossible, are time consuming and tedious processes with chances of high contamination. Instead, the bulk freeze dried (BFD) starter cultures provide convenience, reliability and the ideal inoculum for direct-to-vat inoculation (DVI) and it is the preferred method because of the consistency and security of the culture (Labigne and Kumar 2007). Use of DVI cultures in cheese making is envisaged (Høier et al. 2010). The most commonly used method for the production of concentrated starter cultures is freeze-drying (Palmfeldt and Hahn-Hägerdal 2000). However, more recently Champagne et al. (2012) have optimized the methodology for air-drying of probiotic cultures, which is relatively much cheaper than freeze drying. Innovative techniques in the starter culture preservation

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have been reviewed (Morgan et al. 2006; Kumar and Gandhi 2009). The production of bulk starter cultures devoid of contamination is the corner stone for successful manufacture of fermented products especially of milk based preparations. Addition of the concentrated cultures directly to the vat for product preparation is a breakthrough in the production of fermented foods, which ensures standardization and a high degree of control over the fermentation (Leroy and De Vuyst 2004). Report on the use of DVI cultures for the production of probiotic fermented milks and yoghurt is available (La Torre et al. 2003). High viability of the cultures in BFD starters is important for use in DVI applications (Santivarangkna et al. 2011b).

Use of probiotic or functional cultures along with technical cultures is being practiced to improve the therapeutic or functional properties of the fermented dairy products (De Vuyst 2000; La Torre et al. 2003; Vijayendra and Gupta 2012a; Jadhav et al. 2012; Kaushal and Kansal 2012; Rezaei et al. 2012). The associative behaviour among the probiotic and technical starter cultures in buffalo skim milk has been published recently (Vijayendra and Gupta 2012b).

The composition of milk, in terms of protein, fat and type of triglycerides etc., varies depending on its source. The profile of buffalo milk and cow milk has been recorded (Aneja et al. 2002 and Pandya and Khan 2006). In dairy industry most of the dairy products are manufactured using mixed milk. However, certain products like Cheddar cheese, Mozzarella cheese etc. are invariably prepared from either cow or buffalo milk. Although the performance of lactic cultures in cow milk is well established, very few reports are available on comparative analysis of culture performance among different species of milk (Dutta et al. 1971; Singh and Ranganathan 1978; Khanna and Singh 1979; Rysstad and Abrahamsen 1987). Of the total world milk produced, buffalo milk currently constitutes around 13 % and India is the major producer of buffalo milk (IDF 2008). The advantage and problems (Sindhu 1995) and suitability (Sindhu 1996) of buffalo milk for the processing and preparation of various dairy products have been reported. As a result, technologies should be focused on using buffalo milk for the production of diversified products, including fermented dairy foods, which can meet the national and international requirement (Kumar et al. 2011). To achieve this objective, one needs to know the performance of starter cultures in buffalo milk, along with other sources of milk. Use of buffalo milk has been reported for the preparation of dahi (Jayamanne and Samarajeewa 2010, Vijayendra and Gupta 2012a) and yoghurt (Ghadge et al. 2008, Vijayendra and Gupta 2012a). The scope and applications of BFD cultures can be widened, if these cultures can perform well in milk obtained from various source like cows and buffalos. Very few reports are available on the performance of starter cultures in whole milk (Dutta et al. 1971; Singh and Ranganathan 1978; Khanna and Singh 1979) or

skimmed milk (Vijayendra and Gupta 2012b) of buffalo and currently there are no reports available on the performance of BFD starter cultures of dahi and yoghurt, either alone or in combination with probiotic bacteria, in cow and buffalo milk. Besides, viability and stability, the metabolic activity of the freeze dried cultures is also more important for its use at commercial level. Therefore, in the present work, for the first time, the viability and biochemical performance of BFD starter cultures of dahi and yoghurt, either alone or in combination with probiotic cultures, were evaluated in standardized cow and buffalo milk.

Materials and methods

Preparation of microbial cultures The mesophilic starter cultures of dahi (named as D consisting - *Lactococcus lactis* ssp. *lactis* C10, *Lc. lactis* ssp. *cremoris* C1, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC10, in 1:1:1 ratio), thermophilic yoghurt cultures (named as Y consisting- *Streptococcus salivarius* ssp. *thermophilus* HST and *Lactobacillus delbrueckii* ssp. *bulgaricus* RTS in 1:1 ratio), probiotic strains (*Lactobacillus acidophilus*-R and *Bifidobacterium bifidum*-I) were procured from culture collection center of National Dairy Research Institute, Karnal, India. *Lb. acidophilus*-R and *Bif. bifidum*-I were added to culture combinations of yoghurt (Y) and dahi (D) in the ratio of 1:3 and designated as YAB and DAB, respectively. These cultures were maintained as indicated earlier (Vijayendra and Gupta, 2012b). The culture combinations of D and DAB were grown in 10 L of sterile skim milk yeast autolysate medium (5 % skim milk, 1 % each of yeast autolysate and glucose - pH7.0) at 30 °C and 37 °C, respectively, whereas, culture combinations Y and YAB were grown in 10 L of sterile skim milk tryptone medium (50 % skim milk, 1 % tryptone and 0.1 % glucose-pH7.0) at 37 °C for 18 h. The fermented medium was treated for 10 min with 100 ml of 30 % (w/v) sodium citrate solution to breakdown the curd and the pH of the content was brought to 7.0 using 20 % ammonium hydroxide solution. After 15 min the cells were harvested using a centrifuge (Sharples Centrifuges Ltd, UK) at 20,000 rpm and the cells were subjected to freeze drying using a vacuum freeze drier (model: 30P.2.T, Edwards, UK) after adding a combination of cryoprotective agents (10 % skim milk+1.5 % gelatin+8 % sucrose+1 M monosodium glutamate) at 2 % of growth medium. The freeze dried culture combinations were packed under vacuum in laminated aluminum foil pouches and stored at 4 °C.

Inoculation of buffalo and cow milk samples Freshly collected milk samples of cow and buffalo were standardized to have 4 % fat and distributed in 100 ml quantities in 250 ml Erlenmeyer flasks. After autoclaving at 121 °C for 15 min,

these samples were cooled to room temperature (30 °C). Milk samples were inoculated with 0.01 % (w/v) of freeze dried cultures (having a viable count of 11.03 to 11.45 log₁₀ cfu/ml depending on the combination of cultures as determined by the method given in the following section) of D, DAB, Y and YAB, individually, and mixed thoroughly. The milk samples inoculated with culture combination D was incubated at 30 °C and remaining combinations of cultures (DAB, Y and YAB) were incubated at 37 °C for 14 h and stored at 4 °C until further analysis.

Analysis of dahi/yoghurt samples For viable count determination, the samples were subjected to serial dilution using sterile phosphate buffer. Appropriate decimal dilutions of dahi culture combinations (D and DAB) were spread on yeast dextrose (YD) agar plates, whereas, dilutions of the yoghurt cultures combinations (Y and YAB) were spread on tryptone glucose vegetable (TGV) agar. After solidification, plates containing culture combination D were incubated at 30 °C and rest at 37 °C for 48 h. The viable count was determined and expressed in log₁₀ colony forming units (cfu) per g.

Culture activity (CA) test was performed as per the procedure detailed by Horrall and Elliker (1950) and the results were recorded as % lactic acid. Titratable acidity (TA) was determined by titrating against 0.1 N sodium hydroxide (NaOH) solution (ISI, 1981) and the results were reported as % TA (expressed as lactic acid). Volatile acidity (VA) of the fermented samples was checked as detailed earlier (Vijayendra and Gupta 2012b) following the method of Hempenius and Liska (1968) and the results were expressed as ml of 0.01 N NaOH/50 g of sample. Acetaldehyde and diacetyl content of the yoghurt and dahi samples, respectively, was estimated by the method of Lindsay and Day (1965) and Pack et al. (1968), respectively and the results were indicated in ppm. Proteolytic activity (PA) of the cultured milk was assessed by the method of Hull (1947) and the released tyrosine content was indicated in mg/g sample.

Statistical analysis The results expressed are means of five replicates with standard deviation and the data were analyzed statistically using one way analysis of variance (Snedecor and Cochran 1967).

Results and discussion

Although milk from all the animals has same constituents, the composition varies from species to species. Aneja et al. (2002) compared the profile of the milk collected from cow and buffalo. Similarly, Pandya and Khan (2006) have reviewed the differences in the milk from cow and buffalo.

The technological suitability of milk from various animal species was compared (Barłowska et al. 2011). Buffalo milk has about 11.42 % higher protein than cow milk and buffalo milk fat contains almost double the quantity of high melting triglycerides (9–12 %) than cow milk (5–6 %) (Sindhu 1998). Protein efficiency ratio value of buffalo milk proteins is 2.74 and that of cow milk is 2.49 (Pandya and Khan 2006). With this background, the present study compares the performance of BFD cultures in cow and buffalo milk.

Viability of BFD cultures The viable count of BFD starter cultures of dahi and yoghurt in respective products prepared using standardized buffalo milk is presented in Fig. 1. Significantly ($p < 0.05$) low viable count of probiotic culture combination (DAB) over dahi culture (D) in buffalo milk was noticed, which may be due to the difference in incubation temperature. Unlike the mesophilic dahi culture D, which were grown at 30 °C, the mesophilic cultures present in the combination of DAB might not have grown well at 37 °C, because the incubation temperature of 37 °C is not a favourable temperature for mesophilic lactic cultures. However, significant difference in the viable count of yoghurt cultures obtained in combination Y and YAB could not be seen, as the both the cultures were allowed to grow at 37 °C, which is optimum for yoghurt cultures as well as probiotic cultures. As reported earlier, in buffalo milk (6 % fat), the lactic acid bacteria started to proliferate and reached to a maximum of 6 log₁₀ cfu/ml after 14 h of fermentation in dahi at 26 °C (Jayamanne and Samarajeewa 2010). The mixed yoghurt cultures could take only 4 h in buffalo milk to reach

Fig. 1 Viable count of starter cultures in dahi and yoghurt prepared using standardized buffalo milk D: mesophilic dahi cultures (*Lactococcus lactis* ssp. *lactis* C10, *Lc. lactis* ssp. *cremoris* C1, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* DRC10, in 1:1:1 ratio); DAB: mesophilic dahi cultures (as listed above) in combination with probiotic cultures (*L. acidophilus* R and *B. bifidum* I); Y: thermophilic yoghurt cultures (*Streptococcus salivarius* ssp. *thermophilus* HST and *Lactobacillus delbrueckii* ssp. *bulgaricus* RTS in 1:1 ratio); YAB: thermophilic yoghurt cultures (as listed above) in combination with probiotic cultures (*L. acidophilus* R and *B. bifidum* I). Dahi prepared with culture combination D was incubated at 30 °C and the products made using remaining combinations were incubated at 37 °C

a viable count of approx. $8 \log_{10}$ cfu/ml, whereas, to reach same count it took almost 8 h in cow milk (Khanna and Singh 1979). However, appreciable difference in viable count of yoghurt starter cultures when grown in cow and goat milk was not observed (Rysstad and Abrahamsen 1987).

Biochemical activity of BFD dahi cultures The results of biochemical performance of dahi BFD cultures are presented in Table 1. Dahi culture D was found to be active both in buffalo and cow milk samples with a very close CA factor (0.4 to 0.5 %TA) when subjected to Horrall and Elliker test. Significant difference in the TA among culture combinations D and DAB in any type of milk was not observed. The VA produced by culture combination D was significantly ($p<0.05$) higher in buffalo milk than in cow milk and similar values were seen for culture combination DAB either in cow milk or in buffalo milk. However, the VA produced by culture combination DAB was significantly lower than that produced by culture combination D in both sources of milk. The amount of diacetyl produced by the culture combination D in buffalo milk did not differ significantly with that of cow milk and similar trend was noticed with DAB. However, the diacetyl content produced by DAB was significantly lower than that of D in both types of milk. The trend in PA of cow and buffalo milk dahi samples was comparable to that of diacetyl production with both the combination of cultures. The PA was less in dahi produced by using probiotic culture combination than with that of the combination without probiotic cultures. Over all, there was no difference in the performance of dahi culture combination

DAB either in cow or buffalo milk, after 16 h of incubation at 37 °C. The culture was active both in cow and buffalo milk samples. The TA produced in two types of milk was comparable. The diacetyl and tyrosine contents of dahi made from cow and buffalo milk were in the same order.

Biochemical activity of BFD yoghurt cultures The performance of yoghurt cultures Y and YAB in buffalo milk was comparable to that of cow milk samples (Table 1). Though minor differences in the CA of both the cultures in cow and buffalo milk curds can be observed, these are not of significance. The amount of TA produced in both types of milk was comparable. However, the TA of yoghurt samples fermented with culture combination Y was significantly higher than the TA of probiotic yoghurt (YAB) in both cow and buffalo milk. Similarly, associative growth reduced the production of TA (from 1.68 to 1.44 % lactic acid) by probiotic yoghurt culture combination in buffalo skim milk (Vijayendra and Gupta 2012b). Higher TA is the resultant of higher microbial activity and due to increased production of lactic acid. It results in better coagulation of casein and digestibility of milk protein.

Both the culture combinations (Y and YAB) exhibited slightly more VA production in buffalo milk than in cow milk. Similarly, production of higher CA and VA by parent as well as mutant strain of *L. bulgaricus* in buffalo milk than with cow or goat milk was reported earlier (Singh and Ranganathan 1978). The VA produced by probiotic yoghurt culture combination (YAB) both in cow milk and buffalo milk was higher than that of culture combination Y in these

Table 1 Culture activity and biochemical performance of bulk freeze-dried starter cultures of dahi and yoghurt in standardized buffalo and cow milk

Parameter	Dahi				Yoghurt			
	Buffalo milk		Cow milk		Buffalo milk		Cow milk	
	Culture combinations		Culture combinations		Culture combination		Culture combination	
	D	DAB	D	DAB	Y	YAB	Y	YAB
Culture activity* (% LA)	0.5±0.02 ^a	0.5±0.06 ^a	0.5±0.06 ^a	0.5±0.08 ^a	0.5±0.08 ^a	0.5±0.06 ^a	0.5±0.06 ^a	0.5±0.04 ^a
Titrateable acidity (% LA)	1.0±0.08 ^b	1.2±0.18 ^b	0.9±0.12 ^b	1.1±0.14 ^b	1.6±0.22 ^b	1.5±0.18 ^c	1.6±0.20 ^b	1.3±0.16 ^c
Volatile acidity ^S (ml)	32.5±1.42 ^c	16.1±0.82 ^d	29.2±1.12 ^c	16.0±0.88 ^d	7.9±0.32 ^d	8.6±0.42 ^e	7.8±0.46 ^d	8.5±0.52 ^e
Diacetyl content (ppm)	4.0±0.09 ^f	2.8±0.32 ^g	3.8±0.38 ^f	2.7±0.22 ^g	NA	NA	NA	NA
Acetaldehyde content (ppm) NA	NA	NA	NA	NA	27.5±1.22 ^f	33.2±1.64 ^g	27.2±1.44 ^f	32.8±1.82 ^g
Proteolytic activity (ppm)	0.5±0.05 ^h	0.4±0.02 ⁱ	0.4±0.04 ^h	0.4±0.03 ⁱ	0.4±0.06 ^h	0.3±0.02 ^h	0.3±0.04 ^h	0.3±0.02 ^h

Means (± std. deviation) without a common lower case letter (product wise) within a row are significantly different ($p<0.05$); LA: lactic acid Milk samples (4% fat) containing probiotic cultures (DAB or YAB) were incubated at 37°C and rest at 30°C for 14 h; *by Horrall and Elliker test; ^S: ml of 0.01 N NaOH/ 50 g curd; D: mesophilic dahi cultures (*Lactococcus lactis* ssp. *lactis* C10, *Lc. lactis* ssp. *cremoris* C1, *Lc. lactis* ssp. *lactis* biovar. *diacetylacis* DRC10, in 1:1:1 ratio); DAB: mesophilic dahi cultures (as listed above) in combination with probiotic cultures (*L. acidophilus* R and *B. bifidum* I); Y: thermophilic yoghurt cultures (*Streptococcus salivarius* ssp. *thermophilus* HST and *Lactobacillus delbrueckii* ssp. *bulgaricus* RTS in 1:1 ratio); YAB: thermophilic yoghurt cultures (as listed above) in combination with probiotic cultures (*L. acidophilus* R and *B. bifidum* I); NA: Not applicable

types of milk. Similar observation was made by La Torre et al. (2003) with yoghurt cultures. They have noticed production of very low amount of lactic acid production ($< 9,370 \mu\text{g/g}$) by probiotic lactic cultures (combination of *L. acidophilus* and species of *Bifidobacterium*), when compared to that of typical yoghurt cultures ($> 11,400 \mu\text{g/g}$), whereas, the amount of acetic acid ($> 3,100 \mu\text{g/g}$) produced by same probiotic cultures was several fold high than that of yoghurt cultures ($< 76 \mu\text{g/g}$). This is correlating with the report of Vijayendra and Gupta (2012b), who have also noticed increase in volatile acidity in probiotic yoghurt than in plain yoghurt.

Growth of yoghurt starter cultures was faster in buffalo milk and it produced slightly more acetaldehyde content in buffalo milk rather than in cow milk, which resulted in high organoleptic quality of yoghurt (Singh and Kaul 1982). However, addition of probiotic cultures significantly enhanced the production of acetaldehyde content in both types of milk. Similar trend in buffalo skim milk was reported recently (Vijayendra and Gupta 2012b). Production of low amount (6–9 ppm) of acetaldehyde was noticed in goats' milk than in cows' milk (18 ppm) and similar trend was observed in the production of diacetyl and acetoin by yoghurt starter cultures (Rysstad and Abrahamsen 1987). Addition of threonine (5–10 mg/100 g) increased the acetaldehyde production and it was higher in goat's milk than in cow's milk. However, the amount of acetaldehyde produced in cows' milk without added threonine was significantly higher than in goats' milk without addition of threonine (Rysstad et al. 1990). With regard to PA, significant difference was not observed between yoghurt made with either cow or buffalo milk. Similarly, no difference between Y and YAB cultures either grown in cow milk or buffalo milk was noticed and it is in agreement with a recent report (Vijayendra and Gupta 2012b). However, liberation of more tyrosine in buffalo milk was reported (Singh and Ranganathan 1978) and significantly higher PA in probiotic yoghurt (yoghurt cultures supplemented with either *L. plantarum* CFR 2194 or *L. fermentum* CFR 2192) over the control was noticed (Madhu et al. 2012). This may be due to the variation in culture combinations used or difference in composition of milk of different species (Singh and Ranganathan 1978).

In conclusion, it may be observed that no appreciable difference was noticed in the activity and biochemical performance of all the four culture combinations (D, DAB, Y and YAB) irrespective of the type of milk (cow or buffalo) used. This was in agreement with the findings of Khanna and Singh (1979) that had made similar observation with yoghurt starters in buffalo and cow milk samples. The present finding clearly indicated that the BFD cultures of dahi or yoghurt in combination with probiotic cultures can be used in any type of milk to prepare good quality fermented dairy products.

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